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- (81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NI, NO, NZ, OM, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW.
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- Published:
— with international search report
- For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) Title: METHODS FOR RELATIVE QUANTIFICATION OF SPECIFIC NUCLEIC ACID SEQUENCES

(57) Abstract: This invention relates generally to a method for quantifying the number of occurrences of a specific nucleic acid sequence within a nucleic acid sample in order to circumvent the shortcomings of the methods currently available and to provide reliable quantification of a specific nucleic acid sequence within a nucleic acid sample. The present invention provides a method of assessing an amount of a known target nucleic acid sequence in a sample comprising co-amplifying said target nucleic acid sequence and a known amount of a known control nucleic acid sequence to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence; and determining relative amounts of said respective amplicons by determining relative quantities of a primer extension reaction using each of said respective amplicons as a template.

WO 2004/090160 A1

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference 14187-1PCT	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/CA 03/00547	International filing date (day/month/year) 11/04/2003	(Earliest) Priority Date (day/month/year)
Applicant DNA LANDMARKS INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 5 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

- a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

- b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing:

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☐ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of Invention is lacking** (see Box II).

4. With regard to the **title**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No.

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☒ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/CA 03/00547

Box III TEXT OF THE ABSTRACT (Continuation of Item 5 of the first sheet)

This invention relates generally to a method for quantifying the number of occurrences of a specific nucleic acid sequence within a nucleic acid sample in order to circumvent the shortcomings of the methods currently available and to provide reliable quantification of a specific nucleic acid sequence within a nucleic acid sample. The present invention provides a method of assessing an amount of a known target nucleic acid sequence in a sample comprising co-amplifying said target nucleic acid sequence and a known amount of a known control nucleic acid sequence to produce respective target and control amplicons, wherein said control nucleic acid sequence is different than said target nucleic acid sequence; and determining relative amounts of said respective amplicons by determining relative quantities of a primer extension reaction using each of said respective amplicons as a template.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/00547

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12Q1/68

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C12Q

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

BIOSIS, EPO-Internal, EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PIELBERG G ET AL: "Unexpectedly high allelic diversity at the KIT locus causing dominant white color in the domestic pig" GENETICS, vol. 160, no. 1, January 2002 (2002-01), pages 305-311, XP002265489 ISSN: 0016-6731 the whole document ---	1-39
A	WO 00 63437 A (ILLUMINA INC) 26 October 2000 (2000-10-26) ---	
A	WO 02 20837 A (GARDNER REBECCA ;PYROSEQUENCING AB (SE); EKSTROEM BJOERN (SE); UNI) 14 March 2002 (2002-03-14) --- -/--	



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *G* document member of the same patent family

Date of the actual completion of the international search

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INTERNATIONAL SEARCH REPORT

International Application No

PCT/CA 03/00547

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	BERNARD PHILIP S ET AL: "Real-time PCR technology for cancer diagnostics" CLINICAL CHEMISTRY, vol. 48, no. 8, August 2002 (2002-08), pages 1178-1185, XP002265490 ISSN: 0009-9147 ---	
A	REIS P P ET AL: "Quantitative real-time PCR identifies a critical region of deletion on 22q13 related to prognosis in oral cancer" ONCOGENE 19 SEP 2002 UNITED KINGDOM, vol. 21, no. 42, 19 September 2002 (2002-09-19), pages 6480-6487, XP002265491 ISSN: 0950-9232 ---	
A	ALDERBORN A ET AL: "Determination of single-nucleotide polymorphisms by real-time pyrophosphate DNA sequencing" GENOME RESEARCH, COLD SPRING HARBOR LABORATORY PRESS, US, vol. 10, no. 8, August 2000 (2000-08), pages 1249-1258, XP002218192 ISSN: 1088-9051 ---	
A	RONAGHI M: "PYROSEQUENCING SHEDS LIGHT ON DNA SEQUENCING" GENOME RESEARCH, COLD SPRING HARBOR LABORATORY PRESS, US, vol. 11, no. 1, January 2001 (2001-01), pages 3-11, XP000980886 ISSN: 1088-9051 ---	
T	PIELBERG GERLI ET AL: "A sensitive method for detecting variation in copy numbers of duplicated genes." GENOME RESEARCH, vol. 13, no. 9, September 2003 (2003-09), pages 2171-2177, XP002265492 ISSN: 1088-9051 (ISSN print) ---	1-10
T	QIU P J ET AL: "Quantification of single nucleotide polymorphisms by automated DNA sequencing" BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, ACADEMIC PRESS INC. ORLANDO, FL, US, vol. 309, no. 2, 19 September 2003 (2003-09-19), pages 331-338, XP004451517 ISSN: 0006-291X -----	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

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